

Wild annual *Helianthus anomalus* and *H. deserticola* for improving oil content and quality in sunflower

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Abstract

Within the past decade, the desire for alternative sources of fuels, chemicals, and other industrial materials has received increased attention. Sunflower (*Helianthus annuus* L.) oil has the potential to be improved for nutritional and industrial purposes through selection and breeding. The narrow genetic base of cultivated sunflower has been broadened by the infusion of genes from wild species, resulting in a continuous improvement in agronomic traits. The genus *Helianthus* is comprised of 51 species and 19 subspecies with 14 annual and 37 perennial species. Interest in using wild species in breeding programs has increased, but concerns about the introduction of low oil concentration and quality from the wild species persist. Two annual desert species, *Helianthus anomalus* Blake and *H. deserticola* Heiser, are excellent candidates for increasing oil concentration and enhancing quality based on their adaptation to desert environments. The objective of this study was to collect achenes of *H. anomalus* and *H. deserticola* from the desert southwest USA and assess their potential for improving oil concentration and quality in cultivated sunflower. The sunflower collection took place from 16 to 23 September 2000 and covered a distance of 4100 km in three states: Utah, Arizona, and Nevada. The only *H. deserticola* population collected had an average oil concentration of 330 g/kg, whereas the two populations of *H. anomalus* had an oil concentration of 430 and 460 g/kg, the highest concentration recorded in any wild sunflower species. The linoleic fatty acid concentration in the oil of *H. anomalus* populations was uncharacteristically high for a desert environment, approaching 700 g/kg. A linoleic acid concentration of 540 g/kg in *H. deserticola* was more typical for a desert environment. *H. anomalus* has the largest achenes and the highest oil concentration of any wild sunflower species, and the same chromosome number ($n = 17$) as cultivated sunflower. These features will facilitate the introduction of genes from this wild annual progenitor into cultivated sunflower. The lower saturated fatty acid profile in this species is also a desirable trait offering the potential to reduce saturated fatty acids in cultivated sunflower. Further research will be needed to determine the inheritance of the fatty acids and oil concentration. Other agronomic traits will need to be maintained during the introgression of these traits into cultivated sunflower oil.

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1. Introduction

Interest in potential non-food uses of renewable resources has increased in recent years. Vascular plants produce many compounds and secondary metabolites, one of which is oil. Although oil concentrations of up to 37 g/kg have been reported in whole plants of wild

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sunflower (*H. annuus* L.), the achenes are the primary storage tissue for oil (Seiler et al., 1990). The oil that accumulates in the achenes of wild and cultivated sunflower is composed of triacylglycerols that exist in the liquid form at room temperature and have a low melting point. The fatty acid composition of the achene oil determines its end use suitability.

Sunflower oil is a source of fatty molecules that can be used as reagents for chemical modifications (Girardeau et al., 2000; Leyris et al., 2000). Sunflower oil also has excellent nutritional properties. It is practically free of significant toxic compounds and has a relatively high concentration of linoleic acid. This polyunsaturated fatty acid is an essential fatty acid (not synthesized by humans), and is the precursor of gamma-linolenic and arachidonic acids (Dorrell, 1978). Sunflower oil can be used in the manufacture of lacquers, copolymers, polyester films, modified resins, and plasticizers when there is a price advantage to the manufacturer. The high concentration of linoleic acid and very low concentration of linolenic acid mean that despite the moderate iodine number of 125–140, it has good drying qualities without the yellowing associated with high-linolenic acid oils (Dorrell and Vick, 1997). It can also be used in the manufacture of soap (Suslov, 1968). Emulsifiers and surfactants from fats and oils are already used in formulating pesticides (Pryde and Rothfus, 1989). In addition, with the development of high-oleic sunflower hybrids, sunflower oil has become a more important feedstock for the oleochemical industry, of which the cosmetics industry is a major user (Luhs and Friedt, 1994).

Several reports have been published evaluating sunflower oil and its blends with diesel as a fuel (Morrison et al., 1995). It is estimated that if a farmer in North Dakota, USA, devoted about 10% of their acreage to sunflower production for fuel, the total on-farm fuel requirement could be met (Hofman and Hauck, 1982).

Oil concentration and fatty acid composition, especially oleic and linoleic fatty acids, of oil from wild and cultivated sunflower varies greatly mainly as a response to temperature during seed development (Harris et al., 1978; Seiler, 1986). A high temperature during seed maturation results in oil with high oleic acid concentration, and a low linoleic acid concentration.

The genus *Helianthus* consists of 51 species and 19 subspecies with 14 annual and 37 perennial species (Schilling and Heiser, 1981). The narrow genetic base of cultivated sunflower has been broadened by the infusion of genes from wild species. This has resulted in continuous improvement of agronomic and economic traits in cultivated sunflower (Thompson et al., 1981; Seiler, 1992; Seiler and Rieseberg, 1997). Recent emphasis on

the concentration and fatty acid composition of sunflower oil has increased interest in using wild species in breeding programs, but the introgression of low oil concentration and quality from the wild species into cultivated sunflower is of concern.

H. anomalus (sand sunflower) is a rare endemic species adapted to sand dune and swale habitats in Utah and northern Arizona (Heiser, 1958; Heiser et al., 1969; Thompson et al., 1981; Nabhan and Reichhardt, 1983). Sand sunflower is a diploid annual species of hybrid origin that is endemic to active sand dunes, an extreme environment from its parents, *H. annuus* and *H. petiolaris* (Ludwig et al., 2004). Based on sand sunflower's occurrence in sand dune desert habitats, it frequently has been recognized as drought tolerant with high oil concentration potential, and thus a candidate for improving cultivated sunflower germplasm (Seiler, 1992).

H. deserticola (desert sunflower) is a xerophytic annual species found in sandy soils on the floor of the Great Basin Desert in small populations in western Nevada, west central Utah, and along the border of Utah and Arizona (Heiser et al., 1969). This species is also a diploid hybrid that inhabits the desert floor, an extreme environment relative to its parental species, *H. annuus* and *H. petiolaris* (Gross et al., 2004).

Both species are excellent candidates for oil concentration and quality improvement, as well as drought tolerance. The objective of the study was to undertake an expedition to the desert southwest USA to collect achenes of the two desert species, *H. anomalus* and *H. deserticola*, and assess their potential for improving oil concentration and quality in cultivated sunflower.

2. Materials and methods

2.1. Plant material

Populations of wild sunflowers were collected between 16 and 23 September 2000. The expedition covered a distance of 4100 km in three states: Utah, Arizona, and Nevada. Wild sunflower heads were collected from 200 to 250 plants within each population and bulked into a single sample. Herbarium specimens were deposited at the USDA-ARS wild *Helianthus* herbarium at Fargo, North Dakota. Achene samples were sent to the USDA-ARS North Central Regional Plant Introduction Station, Ames, Iowa, where they will be maintained and distributed.

All populations were collected throughout the broad distributional range of the species. Prior collection sites and generalized distribution maps were used to locate populations. Population size (number and extent), habi-

tat, soil type, achene set per head, and the presence of diseases and insects, and other wild sunflower species located near the collection sites were recorded for each population.

2.2. Oil and fatty acid analyses

Achenes were stored at 5 °C and low humidity (<20%) until analyzed. Each sample represented an isolated, open-pollinated segregating population. Two 6 ml portions from each achene sample were cleaned to remove empty achenes, and analyzed for oil concentration (expressed as a percent on a dry weight basis) by nuclear magnetic resonance (Granlund and Zimmerman, 1975). Fatty acid composition was determined from 10-achenes per sample. A small portion of the pulverized sample (10–20 mg) was transferred to a disposable filter column (Fisher Scientific) and eluted with 3.5 ml of diethyl ether. The oil in the diethyl ether solution was converted to methyl esters using an organic-catalyzed transesterification of the triacylglycerol by the addition of 200 µl of tetramethylammonium hydroxide (10% in methanol), followed by vortexing (Metcalf and Wang, 1981). After 30 min, deionized water was gently added to the reaction mixture, and the upper diethyl ether layer was transferred to a glass vial and capped. The sample was injected into a Hewlett-Packard 5890 gas chromatograph containing a DB-23 capillary column (25 m × 0.25 mm, J & W Scientific), which was held at 190 °C for 5 min, then heated to 220 °C at 10 °C/min, held at 220 °C for 1 min, and then programmed to 240 °C at 20 °C/min, and finally held at 240 °C for 0.5 min, for a total time of 10.5 min. The detector was a flame ionization detector (FID). A fatty acid standard, 21A (Nu-Chek-Prep, Inc.), containing methyl esters of the following acids: palmitic (16:0), stearic (18:0), oleic (18:1), linoleic (18:2), and arachidic (20:0), 11-eicosenoic (20:1), behenic (22:0), and lignoceric (24:0) was used as a reference. Fatty acid peaks were identified by comparing the fatty acid methyl ester

peaks and retention time of the standard with the sample peaks. Chemstation software was used to calculate the area under each peak, and the area of each fatty acid peak was expressed as a percentage of the total area. Fatty acid and oil concentrations were means of two samples per population.

2.3. Data analysis

The data were analyzed using an analysis of variance (ANOVA). Means were separated using Duncan's new multiple range test.

3. Results

Two species, *H. deserticola* and *H. anomalus*, were collected during the exploration (Table 1). It had been 20 years since known populations of these two species were last visited. For unexplained reasons, only one population of *H. deserticola* and two of *H. anomalus* had achene-bearing plants in 2000.

The two populations of *H. anomalus* were found in the northwest part of the species' distributional range. The distance between the two populations was 5 km. One other population of *H. anomalus* with only six plants was found along the east-central edge of the species' range, but it did not have any mature heads with achenes for collection. It had been extremely dry in most of the areas explored with no evidence of the species being present in the fragile sandy habitats.

The only *H. deserticola* population was found in the southeast part of the species' distributional range. No other populations of this species were encountered during the exploration. Again, it had been extremely dry in the fragile sandy habitats of this species' distributional range, with no evidence of the species being present.

The three populations were analyzed for oil concentration and fatty acid composition (Table 2). The *H. deserticola* population had an oil concentration of 330 g/kg, whereas the *H. anomalus* populations had very

Table 1
Species, location, habitat, and size of populations collected during an expedition in Arizona, Utah, and Nevada, 16–23 September 2000

Species	Identification number	Location	Habitat	Population size
<i>Helianthus deserticola</i>	AMES-26094 (DES-2345)	Washington Co., Utah, Anderson Junction	Sandy desert sagebrush	250
<i>H. anomalus</i>	AMES-26095 (ANO-2346)	Jaub Co., Utah, Little Sahara recreation area, White Sands campsite	Shifting white sand dunes	200
<i>H. anomalus</i>	AMES-26096 (ANO-2347)	Jaub Co., Utah, Little Sahara recreation area, Jericho picnic area	Upper slope of shifting white sand dune	250

Table 2

Oil concentration and fatty acid composition of *H. anomalus* and *H. deserticola* populations collected during the exploration in Arizona, Utah, and Nevada

Species	Oil concentration (g/kg)	Fatty acid composition (g/kg)			
		Palmitic (16:0)	Stearic (18:0)	Oleic (18:1)	Linoleic (18:2)
<i>H. deserticola</i> : AMES 26094 (DES-2345)	330 ± 10 a**	65 ± 0.6 a*	35 ± 0.5 a**	320 ± 31 a**	542 ± 31 a**
<i>H. anomalus</i> : AMES 26095 (ANO-2346)	430 ± 0.8 b**	58 ± 4.2 b*	23 ± 1.3 b**	201 ± 5.1 b**	693 ± 1.8 b**
<i>H. anomalus</i> : AMES 26096 (ANO-2347)	460 ± 0.7 c**	64 ± 0.8 a*	23 ± 0.8 b**	212 ± 21.4 b**	686 ± 23.8 b**

Means followed by the same letter in a column are not significantly different at the * $P < 0.05$ and ** $P < 0.005$ level according to the Duncan's new multiple range test.

high oil concentrations, 430 and 460 g/kg, the highest ever reported in any wild sunflower species.

The fatty acid profiles for the two *H. anomalus* populations had a high linoleic acid concentration for a desert environment, approaching 700 g/kg. A lower concentration of 542 g/kg of linoleic acid in *H. deserticola* is more typical of the concentration expected in a desert environment. The oleic acid concentration for *H. deserticola* was 320 g/kg, whereas oleic acid ranged from 201 to 212 g/kg for the two *H. anomalus* populations.

The saturated palmitic and stearic fatty acids in *H. anomalus* averaged 61 and 23 g/kg, respectively, totaling 84 g/kg, about 30% less than typical cultivated sunflower oil with approximately 120 g/kg. The saturated palmitic and stearic acids in *H. deserticola* were 65 and 35 g/kg, respectively, similar to the oil composition of cultivated sunflower.

4. Discussion

The scarcity of plants in the previously documented distributional range of the species is perplexing. Twenty years ago, there were 25 known populations. All these sites were revisited in 2000 with collections possible at only three locations. In 2000, the locations where the plants were found had small populations of 200–250 plants that were scattered over a 0.2 ha area. It had been an extremely dry year in most of the habitats with no evidence of the species being present in the fragile sandy habitats. Another reason for the absence of plants may be because several of the sand dune habitats had become popular off-road vehicle recreational areas, challenging the species' existence in an already fragile habitat.

Wild *Helianthus* populations generally have oil concentrations of 250–300 g/kg, much lower than cultivated sunflower (Seiler, 1985, 1994). The high oil concentration of 460 g/kg in one *H. anomalus* population [AMES 26096 (ANO-2347)] from Jaub County, Utah, was as high as the 440–480 g/kg found in many cultivated

sunflower hybrids (Robertson et al., 1979). Previously, oil concentrations of 315–379 g/kg had been reported for *H. anomalus* populations in Arizona and Utah (Seiler, 1985). The highest oil concentration previously reported for any wild sunflower species was 402 g/kg in a population of *H. niveus* subspecies *canescens* (A. Gray) Heiser (Thompson et al., 1981; Seiler, 1992). Oil concentration of interspecific hybrids can be rapidly increased to an acceptable level by backcrossing with cultivated sunflower lines (Seiler and Rieseberg, 1997). Based on this fact, there should be little concern about the lower oil concentration of the wild species when they are used as sources of genes for agronomic traits.

The linoleic fatty acid concentration observed in the *H. anomalus* populations is unusually high for a hot southern desert location. Generally, high temperatures during flowering, achene filling, and maturation favor a low linoleic acid concentration and a high oleic acid concentration (Seiler, 1986). This relationship is common to both wild and cultivated sunflower. Generally, the cooler northern latitudes have higher levels of linoleic acid in the oil and the warmer southern latitudes have considerably lower linoleic concentrations (De Haro and Fernandez-Martinez, 1991). There is a strong negative relationship between linoleic and oleic acid concentrations, i.e., if one increases, the other decreases (Seiler, 1983, 1986). Thus, the high linoleic concentration of *H. anomalus* is accompanied by a corresponding low concentration of oleic acid near 200 g/kg. The introgression of a stable high (>680 g/kg) concentration of linoleic acid from *H. anomalus* into cultivated sunflower could facilitate expansion of commercial production into the southern latitudes.

The environmental relations between saturated palmitic and stearic fatty acids are less clear than those for linoleic and oleic fatty acids. In a study based on a few wild sunflower species, those collected from northern latitudes had lower saturated fatty acids than those from further south (Seiler, 1994, 1999). The potential to

lower saturated fatty acids in sunflower oil by 30% may be possible by using *H. anomalus*.

Introgression of the wild germplasm characterized in this report into cultivated sunflower should be possible. Molecular phylogenetic evidence indicates that *H. anomalus* and *H. deserticola* are stabilized diploid hybrid derivatives of the two widespread species, *H. annuus* L. and *H. petiolaris* Nutt. (Rieseberg, 1991). Wild annual *H. annuus* and *H. petiolaris* are two of the closest progenitors of cultivated sunflower. These relationships should facilitate the movement of traits from wild annual *H. annuus* and *H. petiolaris* into cultivated sunflower. *H. anomalus* also has larger achenes than any of the other wild sunflower species (Seiler, 1985). All four wild annual species have the same chromosome number ($n=17$) as cultivated sunflower, facilitating the introgression of traits into cultivated sunflower.

5. Conclusions

H. anomalus has larger seed and higher oil concentration than any other wild sunflower species. It also has the same chromosome number as cultivated sunflower. This will facilitate the introgression of agronomic traits from wild germplasm into cultivated sunflower. The lower saturated fatty acid profile in *H. anomalus* has the potential to reduce saturated fatty acids in cultivated sunflower. There appears to be sufficient variability in *H. anomalus* to introduce and select for high linoleic acid concentration and reduced saturated fatty acid concentrations in cultivated sunflower oil.

Further research will be needed to determine the inheritance of the fatty acid composition and oil concentration. Other agronomic traits will need to be maintained during the introgression of these traits into cultivated sunflower. The addition of these wild species populations to the wild sunflower germplasm collection significantly increases the available genetic diversity for improving cultivated sunflower and also insures their future preservation.

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